

CLAIMS

1. Substantially purified glial derived neurotrophic factor.

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2. The substantially purified glial derived neurotrophic factor of claim 1 having a specific activity of at least about 12,000 TU/ μ g.

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3. The substantially purified glial derived neurotrophic factor of claim 1 having a specific activity at least about 24,000 times greater than the specific activity of B49 conditioned medium.

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4. The substantially purified glial derived neurotrophic factor of claim 1 having a molecular weight of about 31-42 kD on non-reducing SDS-PAGE.

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5. The substantially purified glial derived neurotrophic factor of claim 4 comprising a dimeric polypeptide species having a specific activity of at least about 12,000 TU/ μ g.

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6. The substantially purified glial derived neurotrophic factor of claim 1 having a molecular weight of about 20-23 kD on reducing SDS-PAGE.

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7. The substantially purified glial derived neurotrophic factor of claim 6 comprising a monomeric polypeptide sequence.

8. The substantially purified glial derived neurotrophic factor of claim 1 comprising an amino acid sequence as follows:

(Ser)-Pro-Asp-Lys-Gln-Ala-Ala-Ala-Leu-Pro-Arg-Arg-Glu-
(Arg)-Asn-()-Gln-Ala-Ala-Ala-Ala-(Ser)-Pro-(Asp)-
(Asn)

5 9. The substantially purified glial derived
neurotrophic factor of claim 1 comprising the amino
acid sequence of mature rat glial derived neurotrophic
factor as set forth in Fig. 14 (SEQ ID NO:4).

10 10. The substantially purified glial derived
neurotrophic factor of claim 1 comprising the amino
acid sequence of mature human glial derived
neurotrophic factor as set forth in Fig. 19 (SEQ ID
NO:5).

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11. A substantially purified glial derived
neurotrophic factor characterized by the following:

(a) an apparent molecular weight of about 31-42
kD on non-reducing SDS-PAGE;

20 (b) an apparent molecular weight of about 20-23
kD on reducing SDS-PAGE; and

(c) a specific activity of at least about 12,000
TU/mg.

25 12. The substantially purified protein glial derived
neurotrophic factor of claim 11 further characterized
by comprising the amino acid sequence of mature rat
glial derived neurotrophic factor as set forth in Fig.
14 (SEQ ID NO:4)

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13. The substantially purified protein glial derived
neurotrophic factor of claim 11 further characterized
by comprising the amino acid sequence of mature human
glial derived neurotrophic factor as set forth in Fig.

35 19 (SEQ ID NO:5).

14. The substantially purified glial derived neurotrophic factor of claim 11 produced by recombinant DNA technology.

5 15. The substantially purified glial derived neurotrophic factor of claim 1 produced by recombinant DNA technology.

10 16. The substantially purified glial derived neurotrophic factor of claim 15 comprising the amino acid sequence of mature rat glial derived neurotrophic factor as set forth in Fig. 14 (SEQ ID NO:4).

15 17. The substantially purified glial derived neurotrophic factor of claim 15 comprising the amino acid sequence of mature human glial derived neurotrophic factor as set forth in Fig. 19 (SEQ ID NO:5).

20 18. A pharmaceutical composition comprising an effective amount of substantially purified glial derived neurotrophic factor in a pharmaceutically suitable carrier.

25 19. The pharmaceutical composition of claim 18 wherein said factor is comprised of the amino acid sequence of mature rat glial derived neurotrophic factor as set forth in Fig. 14 (SEQ ID NO:4).

30 20. The pharmaceutical composition of claim 18 wherein said factor is comprised of the amino acid sequence of mature human glial derived neurotrophic factor as set forth in Fig. 19 (SEQ ID NO:5).

21. The pharmaceutical composition of claim 18 wherein said factor is produced by recombinant DNA technology.

5 22. The pharmaceutical composition of claim 21 wherein said factor is comprised of the amino acid sequence of mature rat glial derived neurotrophic factor as set forth in Fig. 14 (SEQ ID NO:4).

10 23. The pharmaceutical composition of claim 21 wherein said factor is comprised of the amino acid sequence of mature human glial derived neurotrophic factor as set forth in Fig. 19 (SEQ ID NO:5).

15 24. A method for obtaining substantially purified glial derived neurotrophic factor comprising:
preparing a serum-free growth-conditioned medium of B49 glioblastoma cells;
concentrating the conditioned medium;
20 performing heparin sepharose chromatography on the concentrated conditioned medium;
performing fast protein liquid chromatography or fractions obtained from said heparin sepharose chromatography; and
25 performing reverse-phase high-performance liquid chromatography or fractions obtained from said fast protein liquid chromatography.

25. The method of claim 24 further comprising:
30 subjecting fractions obtained by reverse-phase high performance liquid chromatography to preparative SDS-PAGE; and
performing reverse-phase high-performance liquid chromatography on fractions obtained by preparative
35 SDS-PAGE.

26. A purified and isolated nucleic acid sequence encoding glial derived neurotrophic factor.

27. The nucleic acid sequence of claim 26 comprised
5 of the rat nucleic acid sequence encoding mature rat
glial derived neurotrophic factor as set forth in Fig.
13 (SEQ ID NO:3).

28. The nucleic acid sequence of claim 26 comprised
10 of the human nucleic acid sequence encoding mature
human glial derived neurotrophic factor as set forth
in Fig. 19 (SEQ ID NO:5).

29. A purified and isolated nucleic acid sequence
15 encoding pre-pro glial derived neurotrophic factor.

30. The nucleic acid sequence of claim 28 comprised
of the human nucleic acid sequence encoding pre-pro
rat glial derived neurotrophic factor as set forth in
20 Fig. 13 (SEQ ID NO:3).

31. The nucleic acid sequence of claim 28 comprised
of the human nucleic acid sequence encoding pre-pro
human glial derived neurotrophic factor as set forth
25 in Figures 19 (SEQ ID NO:5) and 22 (SEQ ID NO:8).

32. The nucleic acid sequence of claim 26 encoding
mature rat glial derived neurotrophic factor.

30 33. The nucleic acid sequence of claim 32 encoding
the amino acid sequence of mature rat glial derived
neurotrophic factor as set forth in Fig. 14 (SEQ ID
NO:4).

35 34. The nucleic acid sequence of claim 26 encoding
mature human glial derived neurotrophic factor.

35. The nucleic acid sequence of claim 34 encoding the amino acid sequence of mature human glial derived neurotrophic factor as set forth in Fig. 19 (SEQ ID NO:5).

36. The nucleic acid sequence of claim 26 encoding GDNF selected from the group consisting of:

- (a) a nucleic acid sequence which encodes the amino acid sequence for pre-pro rat GDNF set forth in Figure 13 (SEQ ID NO:3);
- (b) a nucleic acid sequence which encodes the amino acid sequence for mature rat GDNF set forth in Figure 13 (SEQ ID NO:3);
- (c) a nucleic acid sequence which encodes the amino acid sequence for pre-pro human GDNF set forth in Figures 19 (SEQ ID NO:5) and 22 (SEQ ID NO:8);
- (d) a nucleic acid sequence which encodes the amino acid sequence for mature human GDNF set forth in Figure 19 (SEQ ID NO:5);
- (e) a nucleic acid sequence which encodes an amino acid sequence with dopaminergic activity, and said amino acid sequence is recognized by an antibody which binds to a portion of GDNF; and
- (f) a nucleic acid sequence which (1) hybridizes to the complementary sequence of (a), (b), (c) or (d) and (2) encodes an amino acid sequence with dopaminergic activity.

37. A method for preventing or treating nerve damage which comprises administering to a patient in need thereof a therapeutically effective amount of glial derived neurotrophic factor.

38. The method of claim 37 wherein said nerve damage is Parkinson's disease.

39. A pharmaceutical composition for preventing or
treating Parkinson's disease comprising an effective
amount of glial derived neurotrophic factor in a
5 pharmaceutically suitable carrier.

40. A pharmaceutical composition for preventing or
treating damaged or improperly functioning
dopaminergic nerve cells comprising an effective
10 amount of glial derived neurotrophic factor in a
pharmaceutically suitable carrier.

41. A method for preventing damage to or treating
damaged or improperly functioning dopaminergic nerve
15 cells which comprises administering to a patient in
need thereof a therapeutically effective amount of
glial derived neurotrophic factor.

42. A recombinant DNA molecule comprising expression
20 regulatory elements operatively linked to a nucleic
acid sequence encoding glial derived neurotrophic
factor.

43. A host cell transformed with the vector of claim
25 42.

44. A recombinant DNA method for production of glial
derived neurotrophic factor comprising:

(a) subcloning a DNA sequence encoding for glial
30 derived neurotrophic factor into an expression vector
which comprises the regulatory elements needed to
express the DNA sequence;

(b) transforming a host cell with said expression
vector;

(c) culturing the host cells under conditions for amplification of the vector and expression of glial derived neurotrophic factor; and

(d) harvesting the glial derived neurotrophic
5 factor from the host cell culture.

45. The recombinant DNA method of claim 44 wherein said host cell is an animal cell.

10 46. The recombinant DNA method of claim 45 wherein said host cell is COS-7 cells.

47. The recombinant DNA method of claim 44 wherein said host cell is a bacterial cell.

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48. The recombinant DNA method of claim 47 wherein said host cell is E. coli.

49. The recombinant DNA method of claim 48 further
20 comprising the step of refolding the harvested glial derived neurotrophic factor.

50. A recombinant DNA method for production of glial derived neurotrophic factor comprising:

25 (a) culturing the host cell of claim 43 under conditions for amplification of the vector and expression of glial derived neurotrophic factor; and

(b) harvesting the glial derived neurotrophic factor from the host cell culture.

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51. The recombinant DNA method of claim 50 wherein said host cell is an animal cell.

52. The recombinant DNA method of claim 51 wherein
35 said host cell is COS-7 cells.

53. The recombinant DNA method of claim 50 wherein said host cell is a bacterial cell.

54. The recombinant DNA method of claim 53 wherein
5 said host cell is E. coli.

55. The recombinant DNA method of claim 54 further comprising the step of refolding the harvested glial derived neurotrophic factor.

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56. Substantially purified glial derived neurotrophic factor prepared according to the method of claim 24.

57. Substantially purified glial derived neurotrophic
15 factor prepared according to the method of claim 55.

58. Substantially purified glial derived neurotrophic factor prepared according to the method of claim 44.

20 59. Substantially purified glial derived neurotrophic factor prepared according to the method of claim 50.

60. Substantially purified antibodies that recognize glial derived neurotrophic factor.

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61. The antibodies of claim 60 wherein said antibodies are monoclonal.

62. The antibodies of claim 60 wherein said
30 antibodies are polyclonal.

63. A method for preventing or treating nerve damage which comprises implanting cells that secrete glial derived neurotrophic factor into the body of patients
35 in need thereof.

64. The method of claim 63 wherein said patient is suffering from Parkinson's disease.

65. The method of claim 63 wherein said cells are the
5 cells of claim 42.

66. The method of claim 63 wherein said cells are naturally occurring cells that secrete glial derived neurotrophic factor.
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67. The method of claim 63 wherein said cells are maintained within a biocompatible, semipermeable membrane.

15 68. The method of claim 63 wherein said glial derived neurotrophic factor is comprised of the amino acid sequence of mature human glial derived neurotrophic factor as set forth in Fig. 19 (SEQ ID NO:5).

20 69. The method of claim 63 wherein said glial derived neurotrophic factor is comprised of the amino acid sequence of mature rat glial derived neurotrophic factor as set forth in Fig. 14 (SEQ ID NO:4).

25 70. A device for preventing or treating nerve damage by implantation in a patient comprising:

a semipermeable membrane; and

a cell that secretes glial derived neurotrophic factor encapsulated within said membrane,

30 said membrane being permeable to said glial derived neurotrophic factor, and impermeable to factors from said patents detrimental to said cells.

71. The device of claim 70 wherein said cells are the
35 cells of claim 42.

72. The device of claim 70 wherein said cells are naturally occurring cells that secrete glial derived neurotrophic factor.

5 73. The device of claim 70 wherein said glial derived neurotrophic factor is comprised of the amino acid sequence of mature human glial derived neurotrophic factor as set forth in Fig. 19 (SEQ ID NO:5).

10 74. The device of claim 70 wherein said glial derived neurotrophic factor is comprised of the amino acid sequence of mature rat glial derived neurotrophic factor as set forth in Fig. 14 (SEQ ID NO:4).